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Title Page

Report of grant number F49620-92-J-0494

GENE REGULATION IN MEMORY FORMATION AND CIRCADIAN RHYTHMS

Arnold Eskin

Department of Biochemical and Biophysical Sciences

University of Houston

Houston, Texas 77204

23 May 1994

Interim Report for period September 1992 - May 1994

Prepared for Airforce Office of Scientific Research

Bolling AFB, DC, 20332-6448

Technical Report

1. Summary.

One general objective of this research is to investigate the role of gene expression in circadian rhythms and in memory formation. Another general objective of this research is to develop a new system suitable for both biochemical and molecular studies of circadian rhythms. More specifically, having previously identified a number of proteins that may play important roles in memory formation and circadian rhythms, we wished to explore the function of regulation of expression of the genes for these proteins. Our most important progress to date is in developing techniques suitable for measuring changes in gene expression in the Aplysia nervous system (e.g., ribonuclease protection assays) and in developing probes for many Aplysia genes (calmodulin, BiP, porin, HSP-70, ribosomal mRNA, phosphoglycerate kinase, C/EBP, etc.). At this stage, we are at the exciting point where we have just begun to use these techniques and reagents to characterize the regulation of these genes. With regard to the development of model systems for molecular research, we have been unable to observe a circadian rhythm in Halobacteria. We will continue to pursue development of Halobacteria as well as Nematodes and Yeast.

2. Research Objectives.

The work basically has three general objectives: (1) Investigate the regulation and roles of genes for putative oscillator proteins in the eye of Aplysia. (2) Investigate the bacterium, Halobacterium halobium, as a new model system for the molecular biological study of circadian rhythms. (3) Investigate the regulation of genes for proteins that appear to be involved in learning and memory in Aplysia. Specific objectives within each of these broad categories will be discussed under "status of research". The objectives of this research were designed to extend our previous investigations from the biochemical to the molecular biological level. Therefore, new approaches and molecular biological techniques had to be introduced into our research program and laboratory to carry out the research.

3. Status of Research.

The first steps required to accomplish this research were to secure equipment for performing molecular research and hiring some senior investigators with experience using molecular techniques. Within the first 4 months of the project the equipment was obtained but it took longer to hire the personnel. Dr. K. Macphee-Quigley, a Research Associate Professor was hired in September, 1993 and Dr. Tim Liu, a Research Assistant Professor was hired in November, 1993. Both of these individuals have had considerable experience in Molecular Biology (CVs are attached).

A. Objective 1 to investigate genes for putative oscillator proteins.

Our previous research used a physiological screening program to find proteins (putative oscillator proteins) that played important roles in the Aplysia eye circadian pacemaker. Because several lines of evidence indicated the importance of transcription in the circadian oscillator, the goal of these experiments is to determine whether any of the genes of the putative oscillator proteins have characteristics of circadian oscillator components. Our attention focused on studying a heat shock protein called BiP (binding protein, a molecular chaperone) and Porin (a mitochondrial channel protein). Two types of experiments were planned. In one type, the expression of the genes (mRNAs) are investigated in various conditions. In the other type, techniques are being developed to

disrupt the function of the genes. We have made progress primarily in developing techniques to measure gene expression.

Two requirements for measuring gene expression are specific probes to study the mRNA of the gene and techniques for measuring the quantity of the bound probe. With the partial protein sequences we had of BiP and Porin, PCR (the polymerase chain reaction) and gene cloning were used to generate probes. There are several techniques available for measuring levels of mRNAs. Due to the small amount of mRNAs present in eyes, we chose to develop the RNase protection assay to measure levels of mRNAs.

Since BiP was previously cloned in the Kandel lab, probes were made from cDNA clones obtained from Kandel. Using this probe, Costas Koumenis and Dr. Tim Liu have developed the ribonuclease protection assay (RPA) to observe mRNA levels in tissue from 12 eyes. Thus far, BiP mRNA levels do not appear to be affected by either 5-HT or cAMP. Experiments investigating the effect of light on BiP mRNA are presently under way.

To characterize Porin in Aplysia, Dr. Tim Liu and Mark Sloan first obtained the gene sequence of Aplysia Porin. They did this using probes made from human porin to screen Aplysia cDNA libraries. Candidate colonies were found and sequenced. One clone was found to contain the complete coding region for Porin along with both upstream and downstream untranslated regions. Current sequencing of the Aplysia gene shows that it is close to mammalian Porins from various tissues. Riboprobes have been made from coding regions of the gene and are currently being used in ribonuclease protection assays to determine if the mRNA for the gene changes with treatments of serotonin and light given to the Aplysia eye.

Two strategies have been employed by Costas Koumenis to try to "knock out" specific genes in Aplysia. In the first strategy, phosphorothioate double-stranded oligos (S-oligos), which contain the Heat Shock Element (HSE), were added in molar excess to experimental eyes and ganglia. These "artificial" exogenous HSEs should compete with the native HSEs found upstream of the heat-inducible Heat-Shock genes. This competition should result in a decrease in the expression of HS genes and subsequently their proteins in experimental groups compared with heat-shocked but untreated with S-oligo control groups. Such a strategy was successful in the abdominal ganglia of Aplysia where a 70% decrease of HSP expression was achieved. However, under the same experimental conditions, no inhibition of HSP expression was detected in isolated eyes.

The second strategy was developed after the mRNA sequences of the HSPs (BiP, HSP70 and HSC) were published by Dr. Kandel's group. In this case, single-stranded DNA S-oligos were synthesized which have a complementary (antisense) sequence to the mRNA of the targeted protein. Although the exact mechanism of inhibition is not known, it is believed that the DNA:mRNA hybrid is a target for degradation by RNAse H in the cell. This method allows for a more specific targeting of genes, since it can differentially target individual members of the same family (e.g. HSPs). Two such ssDNA oligos which were complementary to 2 non-overlapping sequences at the BiP 5'mRNA region were synthesized. One of the two oligos includes the start of the translation site. Experiments in which a mixture of the two oligos was used in concentrations up to 50μM did not show any significant inhibition of expression of the BiP protein as assayed by 2-dimensional gel electrophoresis. There are reports in the literature that uptake of oligos can be improved by mixing the oligos with agents that can form liposomes (e.g., lipofectin). We are currently in the process of using such agents in order to improve oligo uptake. We are also planning to synthesize oligos to different regions of the mRNA (e.g. just upstream of the translation initiation site), since hybridization efficiencies of oligos to target sequences are sequence specific.

B. Objective 2 to investigate Halobacterium halobium as a model system for molecular circadian studies.

The recent discovery that a prokaryote, cyanobacteria, contains a circadian clock raised the possibility that other prokaryotes might also have clocks available to study. We chose to investigate halobacteria because it was in a different kingdom from cyanobacteria, and it potentially has advantages, e.g., it is not photosynthetic. A graduate student, Mark Sloan, with the help of Dr. Benedik has spent about one year studying habobacteria.

The first question investigated was "does light affect protein synthesis?" To answer this, experiments were performed in which cell cultures, started from a single colony, were grown to various growth phases (early log, log, late log, and stationary) and were then checked for changes in protein synthesis by radioactively labeling newly synthesized proteins with 35S-methionine. Initial experiments were performed by growing cell cultures in both constant darkness and in 12-hour light/dark cycles, adding label to the culture media, exposing the cultures to 3-hour light pulses, processing the cell proteins, and running samples on 2-D polyacrylamide gels (2-D PAGE). No proteins were found whose synthesis was significantly changed by light.

The next question investigated was "is there was a circadian rhythm in overall protein synthesis?" We performed experiments in which cell samples were grown in both 12:12 L/D and in constant D/D and were labeled with 35S-methionine every three hours over a 48-hour period. Total incorporated counts were measured by TCA precipitable counts. The results showed that while there was no conclusive evidence of circadian cycling in protein synthesis, there was some interesting parallel increases/decreases in protein synthesis between cultures grown in both L/D and D/D. To further pursue this, we ran 1-D PAGE gels of these protein samples. When the protein samples from both the L/D and D/D cultures were run on 1-D PAGE, visual analysis showed that the day (subjective day) time samples were noticeably darker than the night (subjective night) time samples. To ensure that temperature changes were not occurring with the light/dark cycles and subsequently causing changes in protein synthesis, we fabricated constant temperature environmental chambers to grow our cell cultures in.

In the next series of experiments, we once again investigated the rhythms of synthesis but used both 2-D and 1-D PAGE of entrained cell samples grown to late log and to stationary growth phases under both L/D and D/D conditions. Samples were taken over a 36-hour period, labeled with 35S-methionine, processed and run on both gel formats. The resulting autoradiograms from these gels showed no significant changes in the synthesis of any specific proteins.

Therefore, the initial investigations of Halobacteria have been disappointing. To date, we have been unable to find proteins whose synthesis is regulated by light and we have been unable to find any evidence for a circadian clock. Our next approach will be to look for rhythms at the molecular level, i.e., rhythmic gene expression. At the same time, we are also investigating two other possible systems, nematodes and yeast.

C. Objective 3 to investigate genes that may be involved in learning and memory.

The experiments of this objective to a large extent parallel those of Objective 1. Thus, to study gene expression, we need to measure mRNA levels and develop the same types of probes and techniques discussed in Objective 1 above. However, an additional feature of Objective 3 is to go beyond the study of mRNA levels by initiating studies of the promoters for genes.

Three projects under this objective are being carried out by Dr. Tim Liu and a new graduate student, Mr. Samer Hattar. In one project, the expression of calmodulin (CaM) is being investigated to confirm and extend results of experiments in which levels of CaM mRNA were shown to change using techniques of *in vitro* translation. Using the RPA assay, the preliminary results indicate that 5-HT regulates the synthesis of CaM mRNA. A second project deals with studying the regulation of BiP at the promoter level. These experiments are intended to help us develop techniques for such studies as well as to study

BiP. To obtain promoter regions of BiP, genomic libraries obtained from the Kandel lab were screened using our BiP probe. So far we have not been able to obtain a BiP clone from this library.- perhaps due to its low titer. Therefore, we now are in the process of making our own Aplysia genomic library. Another project within this objective seeks to find "new" genes that may be regulated during learning. The new technique is called "double - display - reverse - transcriptase - PCR." This project is well underway, but a large effort (time) is required to obtain results using this technique.

4. Articles published and in preparation.

a. Eskin, A., Nunez-Regueiro, M., Noel, F., Homayouni, R., Byrne, J. H., and Zwartjes, R. 1993. Identification of proteins whose mRNA levels are regulated by treatments producing long-term facilitation in Aplysia neurons. Soc. Neurosci. Abst. 19:813.

b. Koumenis, C., Sloan, M., Liu, T., Nunez-Regueiro, M., and Eskin, A. 1994. Investigation of the effects of phase shifting treatments on the regulation of putative oscillator proteins in Aplysia. Soc. Neurosci. Abst. 20 (in press).

c. Zwartjes, R. E., Noel, F., Nunez-Regueiro, M., Homayouni, R., Cook, R., Crow, M. J., Byrne, J. H., Eskin, A. 1994. Treatments producing long-term facilitation affect specific mRNAs and proteins in Aplysia neurons (manuscript submitted, J. Neuroscience).

d. Koumenis, C., Nunez-Regueiro, M., and Eskin, A. 1994. Identification of protein components of the circadian oscillator in the Aplysia eye (in preparation).

e. Koumenis, C., Sloan, M., Liu, T., and Eskin, A. 1994. Regulation of mRNAs for putative circadian oscillator components in the Aplysia eye (in preparation).

5. Professionals involved in Research.

a. Dr. A. Eskin, P. I.
b. Dr. J. H. Byrne, Collaborator
d. Dr. K. Macphee-Quigley, Res. Assoc. Professor
e. Dr. T. Liu, Res. Asst. Professor
f. Mr. Mark Sloan, Graduate Student
g. Mr. Costas Koumenis, Graduate Student

6. Interactions.

The P. I. and several members of the lab presented work at the following meetings and symposia:

a. First Annual Symposium of Center for Biological Timing, "Molecular Basis of Circadian Timing". University of Virginia, Charlottesville, Va. (1992).

b. Meeting of Society for Research on Biological Rhythms, Amelia Island, FI. (1992).

c. EMBO Workshop on Molecular Chronobiology, Leicester, UK (1992).

d. Annual Meeting Society for Neuroscience, Anaheim, CA (1992).

e. Symposium on Connections Between Genetics and Physiology in the study of Biological Clocks, Yamaguchi, Japan (1992).

f. Study Group on Circadian Rhythms sponsored by the FESN, Hotel Pierre, New York, New York (1992).

g. Third International meeting on the Cell and Molecular Neurobiology of Aplysia, Cold Spring Harbor Laboratory, NY (1993).

h. Annual Meeting Society for Neuroscience, Washington, DC. (1993).

i. Gordon Conference on Chronobiology, Session on "Clocks in Culture", Colby-Sawyer College, New London, New Hampshire (1993).

j. Meeting of Society for Research on Biological Rhythms, Amelia Island, FL (1994).

In addition to the above, a one day meeting on Circadian Rhythms was held on Oct. 30, 1993 at the University of Houston to foster communication among scientists working on biological clocks in the Southwest United States. The attendees included students and investigators from labs at Texas A. M. (Drs. Cassone, Hardin, and Golden), at the University of Houston (Eskin, Widger) and from Dr. M. Rea's lab Brooks AFB, San Antonio.

7. Inventions - None.

Curriculum Vitae
Qing-Rong Liu, Ph.D.

I. Identification Data

Name: Qing-Rong Liu, Ph.D., Research Asistant Professor.
Address: Department of Biochemical and Biophysical Sciences,
University of Houston, Houston, TX 77204-5934.
Date of Birth: [redacted]
Place of Birth: [redacted]
Citizenship: P. R. [redacted]
Social Security Number: [redacted]

II. Academic Background

November 1993-Present: Research Assistant Professor, Department of Biochemical and Biophisical Sciences, University of Houston, Houston, TX 77024.

April 1993-November 1993: Senior Molecular Neurobiologist, Hoechst-Roussel Pharmaceuticals Inc. Bridgewater, NJ 08807.

January 1991-February 1993: Postdoctoral Fellow, Roche Institute of Molecular Biology. 340 Kingsland Street, Nutley, NJ 07110.

December 1985-December 1990: Ph.D. Candidate, Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030.
Ph.D. Thesis: Studies of Structural and Functional Relationship of a Major Nucleolar Phosphoprotein - Nucleophosmin/B23.

January 1985-July 1985: Instructor, Division of Cell Biology, Department of Biology, Xiamen University, Xiamen, Fujian, P. R. China.

September 1982-January 1985: M.S. Candidate, Division of Molecular Genetics, Department of Biology, Beijing University, P. R. China.
M.S. Thesis: Heterogeneous Changes of HMG Proteins during Erythropoiesis of Peking Duck and Its Relationship to Gene Activity.

September 1981-September 1982: Research Fellow, Department of Chemistry, First Research Institute of Oceanography, China's Bureau of Oceanography. Qingdao, Shandong, P. R. China.

February 1977-September 1981: Undergraduate Student, Division of Biochemistry, Department of Biology, Beijing University. P. R. China. B.S. Thesis: Purification and Analysis of Bovine Pancreatic Dipeptidase.

III. Academic Fields of Interest

Molecular Biology, Pharmacology, Neuroscience, Biochemistry.

IV. Academic Awards and Membership

Busch Award for Outstanding Graduate Student, 1988.
Member of American Association for the Advancement of Science.

V. Publications

1. Liu, Q-R., Lopez-Corcuera, B., Mandiyan, S., Nelson, H., and Nelson, N. (1993) Cloning and Expression of a Spinal Cord- and Brain-specific Glycine Transporter with Novel Structure Features. J. Biol. Chem. 268, 22802-22808.

2. Liu, Q-R., Lopez-Corcuera, B., Mandiyan, S., Nelson, H., and Nelson, N. (1993) Molecular Characterization of Four Pharmacologically Distinct γ-Aminobutyric Acid Transporters in Mouse Brain. J. Biol. Chem. 268, 2106-2112.

3. Liu, Q-R., Mandiyan, S., Lopez-Corcuera, B., Nelson, H., and Nelson, N. (1993) A Rat Brain cDNA Encoding the Neurotransmitter Transporter with an Unusual Structure. FEBS Lett. 315, 114-118.

4. Liu, Q-R., Lopez-Corcuera, B., Mandiyan, S., Nelson, H., and Nelson, N. (1992) Cloning and Expression of a cDNA Encoding the Transporter of Taurine and β-Alanine in Mouse Brain. Proc. Natl. Acac. Sci. U. S. A. 89, 12145-12149.

5. Lopez-Corcuera, B., Liu, Q-R., Mandiyan, S., Nelson, H., and Nelson, N. (1992) Expression of a Mouse Brain cDNA Encoding Novel GABA Transporter. J. Biol. Chem. 267, 17491-17493.

6. Liu, Q-R., Nelson, H., Mandiyan, S., Lopez-Corcuera, B., and Nelson, N. (1992) Cloning and Expression of a Glycine Transporter from Mouse Brain. FEBS Lett. 305, 110-114.

7. Liu, Q-R., Nelson, H., Mandiyan, S., and Nelson, N. (1992) A Gene Family Encoding Neurotransmitter Transporters. Proc. Natl. Acad. Sci. U. S. A. 89, 6639-6643.

8. Liu, Q-R., and Chan, P. K. (1993) Characterization of Seven Processed Pseudogenes of Nucleophosmin/B23 in the Human Genome. DNA and Cell Biol. 12, 149-156.

9. Liu, Q-R., and Chan, P. K. (1991) Formation of Nucleophosmin/B23 Oligomers Requires Both the Amino and the Carboxyl-Terminal Domains of the Protein. Eur. J. Biochem. 200, 715-721.

10. Liu, Q-R. and Chan, P. K. (1990) Identification of a Long Stretch of Homopurine/Homopyrimidine Sequence in a Cluster of Retroposons in the Human Genome. J. Mol. Biol. 212, 453-459.

11. Chan, P. K., Liu, Q-R., and Durban, E. (1990) The Major Phosphorylation Site of Nucleophosmin (B23) is Phosphorylated by a Nuclear Kinase II. Biochem. J. 270, 549-552.

12. Chan, W-Y., Liu, Q-R., Borjigin, J., Busch, H., Rennert, O.M., Tease, L.A., and Chan, P.K. (1989) Characterization of the cDNA Encoding Human Nucleophosmin and Studies of Its Role in Normal and Abnormal Growth. Biochemistry, 28, 1033-1039.

KATHLEEN MACPHEE-QUIGLEY

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I am a molecular biologist experienced in the analyses of eucaryotic gene expression. I have constructed expression systems as well as isolated and characterized expressed proteins. As a goal-oriented team player, I have acquired significant managerial experience and outstanding problem solving skills.

ACCOMPLISHMENTS

- Performed in depth analyses of the promoter region of malic enzyme gene using nuclear run on assays, northern and southern analyses, electrophorectic mobility gel shift and footprinting assays, transfection analyses and PCR
- Supervised the isolation and sequencing of seven exon containing genomic clones of malic enzyme
- Designed and constructed a baculovirus and E. coli expression systems
- Identified the active site serine of acetylcholinesterase
- Resolved the disulfide bonding pattern of acetylcholinesterase
- Purified and characterized mitochondrial and cytosolic forms of ω-amidase

RESEARCH AND PROFESSIONAL EXPERIENCE

1993-present	Research Associate Professor Department of Biophysical and Biochemical Science University of Houston Cloning, characterization and message analyses of several genes involved in biological clocks and/or learning and memory; teaching molecular biology methods to grad students
1988-1993	Assistant Research Scientist Department of Biochemistry College of Medicine, University of Iowa Laboratory of Dr. Alan Goodridge. Laboratory manager: Responsible for direction of graduate and undergraduate biochemistry students as well as multiple lab assistants. Responsible for budget management and lab organization.
1983-1988	NIH Postdoctoral Research Fellow Department of Chemistry and Division of Pharmacology University of California, San Diego Laboratory of Dr. Susan Taylor in collaboration with Dr. Palmer Taylor.
1977-1983	Research Assistant, School of Life Sciences

University of Nebraska
Graduate student in the laboratory of Dr. Sheldon Schuster.

Education

B.A. with honors, Michigan State University, Sociology

Ph.D., University of Nebraska-Lincoln, Biochemistry and Genetics

Honors

NIH Postdoctoral Fellow
American Heart Association, grant-in-aid
Ad Hoc Reviewer, USDA, Animal Molecular Biology grants panel, 1991-1993
Ad Hoc Reviewer, NIH Study Section, Biochemistry, 1993
Member American Society of Biochemistry and Molecular Biology

Publications

1. **MacPhee-Quigley, K.**, Rapp, T., Klautky, S. and Goodridge, A. (1994) Internal deletion mutants of the malic enzyme promoter result in a higher transcription rate. In preparation.

2. **MacPhee-Quigley, K.** and Goodridge, A. (1994) Okadaic acid inhibits transcription of malic enzyme and fatty acid synthase in chick embryo hepatocytes. In preparation.

3. Chung, S., **MacPhee-Quigley, K.** and Goodridge, A. (1994) The C/EBP-binding region and adjacent sites activate transcription of malic enzyme in chick embryo fibroblasts. In preparation.

4. Radic, Z., Gibney, G., Kawamoto, S., **MacPhee-Quigley, K.**, Bongiorno, C., and Taylor, P. (1992) Expression of Recombinant Acetylcholinesterase in a Baculovirus System: Kinetic Properties of Glutamate 199 Mutants. **Biochemistry** 31: 9760-9767.

5. Gibney, G., Camp, S., Dionne, M,. **MacPhee-Quigley, K.** and Taylor, P. (1990) Mutagenesis of essential functional residues in acetyl-cholinesterase. **PNAS** 87: 7546-7550.

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10. Schumacher, M., Camp, S., Maulet, Y., Newton, M., **MacPhee-Quigley, K.**, Taylor, S.S., Friedmann, T., and Taylor, P. (1986) Primary structure of acetylcholinesterase; Implications for regulation of function. **Fed. Proc.** 45:2976-2981.

11. **MacPhee-Quigley, K.**, Vedvick, T.S., Taylor, P. and Taylor, S., (1986) Active site and other sequence data from Torpedo californica acetylcholinesterase in **Modern Methods of Protein Chemistry**. James L'Italien, editor, Plenum Press, N.Y. 592-598.

12. Schumacher, M., Camp, S., Maulet, Y., Newton, M., **MacPhee-Quigley, K.**, Taylor, S.,Friedmann, T. and Taylor, P. (1986) The primary structure of acetylcholinesterase as deducted from cDNA sequence. **Nature** 319:409-409.

13. **MacPhee-Quigley, K.**, Taylor, P., and Taylor, S. (1985) Primary structures of the catalytic subunits from two molecular forms of acetylcholinesterase: A comparison of NH_2-terminal and active center sequences. **J. Biol. Chem.** 260:12185-12189.

14. **MacPhee, K.**, Nelson, R.E., and Schuster, S.M. (1983) Mutants of Neurospora deficient in asparagine synthetase. **J. Bacteriol.** 156:475-477.

Abstracts

1. MacPhee-Quigley, K., Byrne, J., Arch, S. and Eskin, A. (1994) Cloning and Characterization of a Gene Regulated by Treatments Producing Long-term Facilitation in *Aplysia* Neurons. Neuroscience Abs.
1. MacPhee-Quigley, K., Klautky, S. and Goodridge, A. (1993) Internal Deletions in the Proximal Promoter/Regulatory DNA of the Malic Enzyme Gene Regulate Transcription Rate. Fed. Proc.
2. MacPhee-Quigley, K., Fantozzi, D. and Goodridge, A. (1990) Nuclear proteins bind to the promoter/regulatory region of the chicken malic enzyme gene. Fed. Proc.
3. MacPhee-Quigley, K., Taylor, P., and Taylor, S. (1988) Expression of active, glycosylated acetylcholinesterase using the insect vector baculovirus system. Fed. Proc.
4. MacPhee-Quigley, K., Drotar, A., Vedvick, T., Taylor, P. and Taylor, S. (1987) Characterization of the glycosylation sites of acetylcholinesterase for Torpedo californica. Fed. Proc.
5. MacPhee-Quigley, K., Vedvick, T., Taylor, P. and Taylor, S. (1986) Disulfide bonds in acetylcholinesterase. Fed. Proc.
6. MacPhee-Quigley, K., Vedvick, T., Taylor, P. and Taylor, S. (1985) Active site and other sequence data from Torpedo acetylcholinesterase. Fed. Proc.
7. MacPhee-Quigley, K., Taylor, P. and Taylor S. (1985) Sequence of selected peptides from Torpedo californica acetylcholinesterase. Fed. Proc.
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U N I V E R S I T Y *of* H O U S T O N

Department of Biochemical and Biophysical Sciences

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PROGRAM

South Eastern and Central Texas Society (SECTS) to study Clocks

[SECTS For Clocks]

October 30th, 1993

University of Houston

Houston, Texas

10:00 a.m - 11:30 a.m	Michael Rea
11:30 a.m - 1:00 p.m	Vincent Cassone
1:00 p.m - 2:30 p.m	Lunch and Visit
2:30 p.m - 4:00 p.m	Paul Hardin
4:00 p.m - 4:30 p.m	Susan Golden
4:30 p.m - 5:00 p.m	Bill Widger
5:00 p.m - 5:30 p.m	Arnold Eskin
6:00 p. m	Beer, Wine, BBQ, Eskin's House' 3506 Bellaire Blvd.